Dietary calcium does not exacerbate phytate inhibition of zinc absorption by women from conventional diets[1–4]

Janet R Hunt and Jeannemarie M Beiseigel

ABSTRACT

Background: Although calcium inhibits zinc bioavailability in rats, especially from high-phytate diets, the effect of calcium on zinc absorption by humans from practical diets remains unclear.

Objective: The objective was to test the inhibitory effect of dietary calcium, in Western diets with high and low phytate content, on zinc absorption.

Design: Using a 2 × 2 factorial design, zinc absorption was determined in 10 healthy women from 1-d diets with moderate and high calcium contents of ≈700 and 1800 mg/d and low and high phytate contents of ≈440 and 1800 mg/d. Absorption was measured by using extrinsically added ^{65}Zn and subsequent whole-body scintillation counting.

Results: Mean (±SE) fractional zinc absorption was 32.8 ± 2.3% from the moderate-calcium, low-phytate diet; 26.9 ± 2.4% from the moderate-calcium, high-phytate diet; 39.4 ± 2.4% from the high-calcium, low-phytate diet; and 26.2 ± 2.3% from the high-calcium, high-phytate diet. The respective values for absolute zinc absorption were 3.8 ± 0.3, 3.0 ± 0.3, 4.5 ± 0.3, and 3.2 ± 0.3 mg/d. Phytate significantly reduced fractional zinc absorption by ≈10 percentage points and reduced absolute zinc absorption by 25%, or ≈1 mg/d. Differences in dietary calcium did not affect zinc absorption, regardless of a high or low dietary phytate content.

Conclusions: In healthy women consuming 1-d menus of ordinary foods (some fortified with calcium), dietary phytate reduces zinc absorption, but calcium does not impair zinc absorption, regardless of whether dietary phytate is low or high. *Am J Clin Nutr* 2009;89:839–43.

INTRODUCTION

Both phytate (used here as a general term for phytic acid, inositol hexaphosphate, or 6-phosphoinositol) and calcium are commonly listed as inhibitors of zinc absorption (1, 2). However, calcium has been hypothesized to be especially inhibitory in the presence of phytate, because 1:1:1 or 2:1:1 molar concentrations of calcium, zinc and phytate, respectively, are much less soluble than similar concentrations of zinc and phytate only when tested at pH levels typical of the small intestine (3). Fordyce et al (4) reported that zinc bioavailability, measured from growth and tibia zinc concentrations of rats, was better predicted by the (phytate × calcium):zinc ratio than the phytate:zinc ratio.

Controlled human studies addressing the effects of calcium, with or without phytate, on zinc bioavailability are limited. Dietary phytate reduces zinc absorption in single-meal isotopic tracer studies (5–7) and, together with dietary zinc content, is a primary predictor of human zinc absorption in algorithm models based on complete (≥1 d) diets (8, 9). Calcium added to low-phytate meals does not impair zinc absorption, as measured by using isotopic tracers (10–13). Consistent with the theory that calcium interacts with phytate to inhibit zinc absorption, Sandström and Cederblad (14) reported decreased zinc absorption with the addition of milk to a soybean-based meal. However, in a separate report, Sandström et al (15) described enhanced zinc absorption from a meal based on whole-meal bread when calcium was added in the form of milk products; this enhancement was attributed to the protein in the milk products. Lonnerdal et al (11) also reported increased rather than decreased zinc absorption by adults when infant soy formula was supplemented with calcium; the authors suggested that the additional calcium might bind phytate, freeing zinc for absorption.

In summary, whereas substantial evidence documents the inhibitory effect of phytate on zinc absorption, the effect of dietary calcium or of a phytate × calcium interaction is much less clear. The latter effects have not been tested in human isotopic studies using commonly consumed foods. The study reported here tested the effects of dietary calcium and phytate in a 2 × 2 factorial design using Western diets formulated from ordinary foods, and the absorption of zinc isotope was measured by using a whole-body scintillation counter.

SUBJECTS AND METHODS

Subjects

A power analysis was conducted to determine the number of volunteers required to detect a statistically significant (α = 0.05) calcium × phytate interaction. On the basis of variability from

[1] From the US Department of Agriculture–Agricultural Research Service, Grand Forks Human Nutrition Research Center, Grand Forks, ND.

[2] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the US Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

[3] Supported by the USDA Agricultural Research Service.

[4] Reprints not available. Address correspondence to JR Hunt, USDA-ARS GFHNRC, 2420 2nd Avenue N, STOP 9034, Grand Forks, ND 58202-9034. E-mail: janetrhunt@gmail.com.

Received October 29, 2008. Accepted for publication December 9, 2008.
First published online January 28, 2009; doi: 10.3945/ajcn.2008.27175.

similar research conducted by our laboratory (16), 8 women were required to detect a difference in zinc absorption of 7 percentage points with a power of 90%. To reduce the possible influence of heterogeneity in this small of a sample, the sample size was rounded up to achieve study completion by 10 women. Women were recruited via local advertising and met eligibility requirements if they were aged 21–50 y, with no apparent disease as indicated by brief questionnaires, interviews, and measures of blood pressure, blood glucose, hemoglobin, creatinine, and liver transaminases. Weight-for-height [body mass index (BMI; in kg/m^2)] was between the 5th and 95th percentiles for the US population of similar age. The women had not been pregnant within the previous year and were not lactating. Subjects had not regularly used zinc supplements exceeding 20 mg/d or calcium supplements exceeding 1000 mg/d, and they agreed to discontinue all nutrient supplements when their application was submitted, generally 4–8 wk before the beginning of the study. None of the subjects routinely used medication, with the exception that routine use of hormonal contraceptives or hormone replacement therapy was allowed. The 10 subjects who completed the study had a mean ($\pm$SD) age of 35 $\pm$ 10 (range: 21–47) y and BMI of 26 $\pm$ 3 (range: 23–32).

All procedures and potential risks were explained, and all subjects gave written informed consent. This study received approval for human subjects from the University of North Dakota's Institutional Review Board and its Radioactive Drug Research Committee and from the US Department of Agriculture's Radiological Safety Committee.

Dietary treatments

The effects of calcium and phytate on zinc absorption were assessed by using 2 levels of each in a 2 $\times$ 2 factorial design. Zinc absorption was measured from 4 different 1-d menus (**Table 1**), all of which were consumed by each subject in random order. The experimental menus contained $\approx$11.5 mg Zn, a moderate ($\approx$700 mg/d) or high ($\approx$1800 mg/d) amount of dietary calcium, and a low ($\approx$440 mg/d) or high ($\approx$1800 mg/d) amount of phytic acid (**Table 2**). All the foods were commercially available, and none were fortified with zinc. The calcium content of the menus was increased by modifying serving sizes and by replacing unfortified foods with similar foods that were commercially fortified with calcium. Phytate was modified by the isocaloric substitution of whole grain, nut, and legume products for some meat and refined-grain products. The increases in calcium and phytate were approximately evenly distributed between the 3 meals, although the increase in phytate at the lunch meal was less than planned because subsequent measurement indicated considerably less phytate in wild rice than has been reported (17). Each menu provided 2200 kcal and $\approx$85 g protein. The high- and low-phytate diets contained 32 and 19 g/d dietary fiber, respectively. Foods were weighed to 1% accuracy and consumed quantitatively. City water was consumed as desired, after analyses indicated minimal zinc content.

Zinc absorption measurements

Zinc absorption was measured by using ^{65}Zn radiotracer and whole-body scintillation counting methods. The study lasted 16 wk, with an absorption measurement every 4 wk. The subjects consumed a 1-d experimental menu for 2 consecutive days. On the second day, the entire menu (3 meals) was extrinsically labeled with 7.4 kBq (0.2 μCi) ^{65}Zn tracer. The tracer was added to one of the primary sources of zinc in each meal, and the specific activity (ratio of ^{65}Zn to elemental zinc) was constant

TABLE 1
Menus for the experimental diets[1]

Moderate-calcium, low-phytate diet	High-calcium, low-phytate diet	Moderate-calcium, high-phytate diet	High-calcium, high-phytate diet
Breakfast			
Orange juice	Orange juice + calcium	Orange juice	Orange juice + calcium
Skim milk	Skim milk + calcium	Skim milk	Skim milk
White bagel	White bagel	Wheat bagel	Wheat bagel
Cream cheese	Cream cheese	Peanut butter	Peanut butter
Ham slice	Ham slice	Shredded wheat + sugar	Shredded wheat + sugar
Strawberries	Strawberries	Strawberries	Strawberries
Lunch			
Grape juice	Grape juice + calcium	Grape juice	Grape juice + calcium
Skim milk	Skim milk + calcium	Skim milk	Skim milk + calcium
Potato casserole with beef, green beans, gravy	Potato casserole with beef, green beans, gravy	Wild rice casserole with beef, green beans, gravy	Wild rice casserole with beef, green beans, gravy
Refined pasta salad with vegetables	Refined pasta salad with vegetables	Wheat pasta salad with vegetables, navy beans	Wheat pasta salad with vegetables, navy beans
Carrots	Carrots	Carrots	Carrots
Peaches	Peaches	Peaches	Peaches
Supper			
Roast beef, white bun	Roast beef, white bun	Turkey, whole-wheat bun	Tuna, whole-wheat bun
Salad dressing	Salad dressing	Salad dressing	Salad dressing
Romaine lettuce, tomato	Romaine lettuce, tomato	Romaine lettuce, tomato	Romaine lettuce, tomato
Tortilla chips	Tortilla chips	Tortilla chips	Tortilla chips
Pears	Pears	Pears, oatmeal, and almonds	Pears, oatmeal, and almonds
Skim milk	Skim milk + calcium		Skim milk + calcium
Shortbread cookies	Shortbread cookies		

[1] Menus varied in type as well as in portion sizes. For instance, the high-calcium menus included milk in larger portions and were calcium-fortified.

TABLE 2

Zinc absorption in women as affected by phytate and calcium in conventional diets providing 2200 kcal and ≈85 g protein[1]

Diet	Zinc	Calcium	Phytate	Phytate:zinc molar ratio	Phytate × calcium:zinc millimolar ratio	Zinc absorbed[2]	Zinc absorbed[3]
	mg/d	*mg/d*	*mg/d*			*%*	*mg*
Moderate-calcium, low-phytate	11.5 ± 0.4[4]	669 ± 21	483	4.2	69	32.8 ± 2.3	3.8 ± 0.3
Moderate-calcium, high-phytate	11.3 ± 0.0	697 ± 26	1781	15.6	272	26.9 ± 2.4	3.0 ± 0.3
High-calcium, low-phytate	11.4 ± 0.2	1897 ± 44	391	3.4	161	39.4 ± 2.4	4.5 ± 0.3
High-calcium, high-phytate	12.2 ± 0.3	1719 ± 74	1789	14.5	623	26.2 ± 2.3	3.2 ± 0.3

[1] Dietary minerals were analyzed in triplicate. Daily phytate intake was calculated from analyses of each meal, with an average CV of 2.3% for replicate determinations. For absorption measurements, n = 10. The diet sequence did not significantly influence the results (ANOVA).

[2] ANOVA: calcium effect (P = 0.17), phytate effect (P = 0.0002), calcium × phytate effect (P = 0.08).

[3] ANOVA: calcium effect (P = 0.09), phytate effect (P = 0.0005), calcium × phytate effect (P = 0.3).

[4] Mean ± SE (all such values).

for all meals. All meals were served at the research center. During the 4 wk between each 2-d set of controlled diets, the subjects consumed their self-selected diets.

Absorption was determined by serial whole-body scintillation counting; individual retention curves were used to correct for endogenous ^{65}Zn excretion (18). Whole-body radioactivity was determined before the labeled meals, after the second labeled meal, and twice weekly thereafter. The initial total body activity (representing 100% of the administered dose) was calculated from the whole-body activity after 2 labeled meals (before any unabsorbed isotope was excreted), divided by the fraction of the total activity contained in those 2 meals. The percentage absorption was determined by extrapolating back to the time of isotope administration along the linear portion (days 14–28 after ^{65}Zn administration) of a semilogarithmic retention plot (the natural logarithm of percentage remaining radioactivity versus time). The next absorption determination was corrected for previously administered ^{65}Zn by subtracting the background whole-body radiation (measured 1–2 d before the meals) from all subsequent whole-body counting measurements for that treatment.

Chemical analyses of diets

Duplicate diets were prepared for analysis with precautions to avoid trace mineral contamination. Diet samples were analyzed for calcium and zinc contents by inductively coupled argon plasma emission spectrophotometry after nitric acid and hydrogen peroxide digestion. Analytic accuracy was monitored by assaying a typical diet standard (SRM 1548a; US National Institute of Standards and Technology, Gaithersburg, MD), yielding mean (±SD) results that were 94 ± 5% of certified values for calcium and 99 ± 9% of certified values for zinc. Dietary phytate was determined by acid-extraction, ion-exchange separation, and phosphorus analysis (19) and quantified by assuming 6 mol P/mol phytic acid.

Statistics

The effects of dietary treatment and equilibration time were determined by using 2-factor repeated-measures analysis of variance (ANOVA), with individual volunteers serving as their own controls (20). ANOVA results were considered significant if the P value was <0.05.

RESULTS

Fractional zinc absorption was significantly lower with high dietary phytate (Table 2). Fractional zinc absorption averaged ≈10 percentage points lower with the high- than with the low-phytate diet. A tendency for this effect to be more pronounced when dietary calcium was high was not statistically significant (P = 0.08 for the interaction).

Absolute zinc absorption was 25% lower with the high- than with the low-phytate diet, an absorption difference of 1 mg/d. Dietary calcium did not significantly affect absolute zinc absorption; a possible trend suggested by the analysis (P = 0.09 for the main effect of calcium) favored greater, rather than lower, zinc absorption when dietary calcium was high (Table 2).

DISCUSSION

The present results confirm the negative effect of phytic acid on zinc absorption (5–9). With an increase in the phytate:zinc molar ratios from ≈4 to 15, the amount of zinc absorbed from a 1-d diet was reduced by 25%, or 1 mg. Current dietary recommendations that emphasize greater consumption of phytate sources such as whole grains, legumes, and nuts would likely reduce dietary zinc bioavailability, although not as much as with the present experimental diets. We estimate that a phytate:zinc ratio of ≈8 would result from current US recommendations that include increased consumption of whole grains (estimated by calculation using MyPyramid menus; 21). Whereas the phytate:zinc molar ratio of 15 in the high-phytate diets in the present study probably exceeds that of most Western diets, phytate:zinc molar ratios ≥15 are more likely for vegetarians and are common in developing countries (8), where zinc deficiency is a concern. Ellis et al (22) recommended ratios of ≤10 to obtain adequate zinc bioavailability from human diets and reported average dietary phytate:zinc molar ratios of ≈8 for US omnivorous women, ≈14 for US vegetarian women, ≈13 for Asian Indian vegetarians, and ≈20 for Nepalese vegetarians. Phytate:zinc molar ratios have been estimated to be as high as 26 in countries of South Asia and sub-Saharan Africa (8).

The lack of a significant effect of dietary calcium or of a phytate × calcium interaction on zinc absorption in the present study is reassuring, especially with the emphasis in recent years on increased calcium ingestion from food and supplements in

TABLE 3
Comparison of measured zinc absorption with that predicted from daily dietary zinc and phytate (but not calcium) contents by using proposed absorption models[1]

Diet	Observed zinc absorption[2]	Predicted zinc absorption, IZiNCG (8)	Predicted, Miller et al (9)	Predicted, modified Hunt et al (25)
	mg	*mg (% of observed)*	*mg (% of observed)*	*mg (% of observed)*
Moderate-calcium, low-phytate	3.8 ± 0.3	3.54 (93)	3.64 (96)	4.02 (106)
Moderate-calcium, high-phytate	3.0 ± 0.3	2.58 (86)	2.49 (83)	3.05 (102)
High-calcium, low-phytate	4.5 ± 0.3	3.68 (82)	3.74 (83)	4.10 (91)
High-calcium, high-phytate	3.2 ± 0.3	2.73 (85)	2.63 (82)	3.20 (100)

[1] IZiNCG, International Zinc Nutrition Consultative Group.
[2] $n = 10$.

response to evidence that calcium increases bone mineral density (23). In the United States, calcium has been commercially added to fortify multiple foods, including milk, juice, and grain products, and calcium is commonly ingested in the form of dietary supplements or antacids, especially among the elderly (24).

Ellis et al (22) recommended phytate × calcium:zinc millimolar ratios of ≤200 to obtain adequate zinc bioavailability from human diets. Although both the high-phytate diets of the present study exceeded this criterion (millimolar ratios of 272 and 623), and were associated with significantly lower zinc absorption (Table 2), there was no evidence that calcium contributed to the inhibition of zinc absorption by phytate. Although the effects of calcium were nonsignificant in this study, the direction of the results indicated that if there was any effect, calcium tended to improve rather than inhibit zinc absorption.

Currently proposed models to predict zinc absorption from human diets are based on dietary zinc and phytate, but not on calcium content. The predictive power of the International Zinc Nutrition Consultative Group logit regression model (8) was not improved by inclusion of calcium in the model. Likewise, Miller et al (9), using data from 14 absorption studies, found no relation between the residuals from their model predictions and either the dietary calcium or the calcium × phytate:zinc millimolar ratio. When these models were applied to predict zinc absorption in the present study, the International Zinc Nutrition Consultative Group model (8) predicted 93%, 86%, 82%, and 85%, the model of Miller et al (9) predicted 96%, 83%, 83%, and 82%, and our modification of the Miller et al model (25) predicted 106%, 102%, 91%, and 100% of the observed results, respectively, for the moderate-calcium and low-phytate diet, the moderate-calcium and high-phytate diet, the high-calcium and low-phytate diet, and the high-calcium and high-phytate diet (**Table 3**). If high dietary calcium inhibited zinc absorption primarily with high dietary phytate, one would expect the last of each set of predictions to overestimate, rather than underestimate, the observed results. The predictions by these models do not appear to be biased by not including a possible inhibitory effect of dietary calcium on zinc absorption.

Knowing the effect of dietary bioavailability on zinc absorption is important for assessing the susceptibility of people, especially those in developing countries, to zinc deficiency. Because no reliable biochemical or clinical markers of marginal zinc status are available, the risk of zinc deficiency in a population may best be evaluated by assessing the usual dietary zinc content and bioavailability. The current results are consistent with those of other studies (8, 9), which concluded that the phytate and zinc contents of the diet are the primary dietary factors that predict zinc absorption. For populations at risk of inadequate bioavailable zinc, successful interventions may require both increases in dietary zinc content as well as reductions in dietary phytate.

In conclusion, in healthy women consuming 1-d diets nutritionally modified using foods commercially available in the United States, zinc absorption was inhibited by ≈25% by high dietary phytate. However, zinc absorption from these diets was not affected by increased calcium ingestion, achieved partly by using calcium-fortified foods, even when dietary phytate was also high.

We gratefully acknowledge the valuable contributions of other members of our human studies research team: Bonnie Hoverson (supervised the planning and preparation of the experimental diets), Emily J Nielsen (managed volunteer recruitment and coordination), Sandy K Gallagher (supervised the clinical laboratory analyses), Carol Zito (managed the food radiolabeling), Jackie Nelson (conducted the whole-body counting of volunteers), Glenn I Lykken (consulted on the use of the whole-body counter), and LuAnn Johnson (conducted the statistical analyses).

The authors' responsibilities were as follows—JRH: initiated the experiment, drafted the manuscript, and assisted in the planning and implementation of the study and in the data analysis; and JMB: planned and conducted the experiment and assisted with the data analysis and manuscript preparation. Neither of the authors had any personal or financial conflict of interest.

REFERENCES

1. Food and Nutrition Board. Institute of Medicine. Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. Washington, DC: National Academy Press, 2001.
2. World Health Organization. Trace elements in human nutrition and health. Geneva, Switzerland: WHO, 1996:72–104.
3. Oberleas D, Muhrer ME, O'Dell BL. Dietary metal-complexing agents and zinc availability in the rat. J Nutr 1966;90:56–62.
4. Fordyce EJ, Forbes RM, Robbins KR, Erdman JW Jr. Phytate x calcium/zinc molar ratios: are they predictive of zinc bioavailability? J Food Sci 1987;52:440–4.
5. Navert B, Sandström B, Cederblad A. Reduction of the phytate content of bran by leavening in bread and its effect on zinc absorption in man. Br J Nutr 1985;53:47–53.
6. Sandström B, Almgren A, Kivisto B, Cederblad A. Zinc absorption in humans from meals based on rye, barley, oatmeal, triticale and whole wheat. J Nutr 1987;117:1898–902.
7. Sandström B, Sandberg AS. Inhibitory effects of isolated inositol phosphates on zinc absorption in humans. J Trace Elem Electrolytes Health Dis 1992;6:99–103.

8. International Zinc Nutrition Consultative Group. Assessment of the risk of zinc deficiency in populations and options for its control. In: Hotz C, Brown KH, eds. Food and Nutrition Bulletin. Tokyo, Japan: United Nations University Press, 2004:S96–203.
9. Miller LV, Krebs NF, Hambidge KM. A mathematical model of zinc absorption in humans as a function of dietary zinc and phytate. J Nutr 2007;137:135–41.
10. Spencer H, Vankinscott V, Lewin I, Samachson J. Zinc-65 metabolism during low and high calcium intake in man. J Nutr 1965;86:169–77.
11. Lonnerdal B, Cederblad A, Davidsson L, Sandström B. The effect of individual components of soy formula and cows' milk formula on zinc bioavailability. Am J Clin Nutr 1984;40:1064–70.
12. Dawson-Hughes B, Seligson FH, Hughes VA. Effects of calcium carbonate and hydroxyappetite on zinc and iron retention in postmenopausal women. Am J Clin Nutr 1986;44:83–8.
13. Wood RJ, Zheng JJ. Milk consumption and zinc retention in postmenopausal women. J Nutr 1990;120:398–403.
14. Sandström B, Cederblad A. Zinc absorption from composite meals II. Influence of the main protein source. Am J Clin Nutr 1980;33:1778–83.
15. Sandström B, Arvidsson B, Cederblad A, Bjorn-Rasmussen E. Zinc absorption from composite meals. I. The significance of wheat extraction rate, zinc, calcium, and protein content in meals based on bread. Am J Clin Nutr 1980;33:739–45.
16. Hunt JR, Matthys LA, Johnson LK. Zinc absorption, mineral balance, and blood lipids in women consuming controlled lactoovovegetarian and omnivorous diets for 8 wk. Am J Clin Nutr 1998;67:421–30.
17. Harland BF, Oberleas D. Phytate in foods. World Rev Nutr Diet 1987; 52:235–59.
18. Hunt JR, Gallagher SK, Johnson LK, Lykken GI. High- versus low-meat diets: effects on zinc absorption, iron status, and calcium, copper, iron, magnesium, manganese, nitrogen, phosphorus, and zinc balance in postmenopausal women. Am J Clin Nutr 1995;62:621–32.
19. Association of Official Analytical Chemists. Phytate in foods (method 986.11). In: Helrich K, ed. AOAC official methods of analysis. 15th ed. Arlington, VA: Association of Official Analytical Chemists, 1990.
20. SAS Institute Inc. SAS OnlineDoc 9.1.3. Cary, NC: SAS Institute Inc, 2004.
21. US Department of Agriculture. MyPyramid.gov: steps to a healthier you. Sample menus for a 2000 calorie food pattern. Available from: http://www.mypyramid.gov/downloads/sample_menu.pdf (cited 14 May 2007).
22. Ellis R, Kelsay JL, Reynolds RD, Morris ER, Moser PB, Frazier CW. Phytate: zinc and phytate x calcium:zinc millimolar ratios in self-selected diets of Americans, Asian Indians, and Nepalese. J Am Diet Assoc 1987;87:1043–7.
23. Food and Nutrition Board, Institute of Medicine. Dietary reference intakes for calcium, phosphorus, magnesium, vitamin D, and fluoride. Washington, DC: National Academy Press, 1997.
24. Ervin RB, Kennedy-Stephenson J. Mineral intakes of elderly adult supplement and non-supplement users in the third national health and nutrition examination survey. J Nutr 2002;132:3422–7.
25. Hunt JR, Beiseigel JM, Johnson LK. Adaptation in human zinc absorption as influenced by dietary zinc and bioavailability. Am J Clin Nutr 2008;87:1336–45.